TRIGLYCERIDE

En

REF 08V5525

REF 08V5520

Alinity c

Diagnostic reagent for the determination of Triglyceride concentration.
Liquid. Mono reagent. Store at +2/+8°C. For In Vitro Diagnostic use. **Do not freeze.**
The products with 08V5525/08V5620 reference numbers provided are compatible for use with Abbott Alinity biochemistry autoanalyzer series.

Changes made in the instructions for use are marked as gray.

The instructions must be followed carefully. The reliability of the test results cannot be guaranteed unless these instructions are followed.

NAME

Triglyceride

INTENDED USE

Triglyceride assay is used for the quantitative determination of Triglyceride concentration in human serum and plasma on analyzer.

SUMMARY AND EXPLANATION OF THE TEST

The simplest lipids composed of fatty acids are triacylglycerols (TAGs), also known as triglycerides (TG), fats or neutral fats. TG consists of three fatty acids, each linked to a single glycerol by an ester bond.[1] More than 90% of the daily lipid intake by adults is TG. The rest of the dietary lipids consist mainly of cholesterol, cholesteryl esters, phospholipids and non-esterified ("free") fatty acids. Digestion of lipids begins in the stomach, catalyzed by a lipase (lingual lipase) that originates from glands on the back of the tongue. TG molecules, especially those containing fatty acids of short or medium chain length (less than 12 carbons, as found in milk fat) are the primary target of this enzyme. Similar TGs are also broken down by a separate gastric lipase secreted by the gastric mucosa. The major absorption of TG molecules occurs by the mucosal cells of the intestinal villi and prior to the absorption, it is hydrolyzed by an esterase enzyme called pancreatic lipase to remove fatty acids, preferably at carbons 1 and 3. As a result, the main products of hydrolysis are 2-monoacylglycerol and free fatty acids.[2] As a result, dietary TGs are absorbed from the small intestine and secreted into the lymphatic system. They then pass into the systemic circulation as chylomicrons (CMs) via the ductus thoracicus. Muscle and adipose tissue separate some of the TGs from the chylomicrons and then, the liver removes the chylomicron residues and converts them into cholesterol-rich lipoproteins. Although most of the TGs in the blood are absorbed from the small intestine, the liver also produces and releases some TGs into the blood. Apolipoproteins are proteins that help in the binding, transport and metabolism of lipids and also interact with TGs. Damage to the structure of these proteins or related enzymes may lead to clinical dyslipidemia.[3]

Specialized cells called adipocytes or fat cells store large amounts of TGs in the form of fat droplets that almost fill the cell. When energy is needed, the TGs stored in adipocytes are hydrolyzed by lipase enzymes and transported as free fatty acids to sites in the body where they are needed as fuel. An advantage of using TGs instead of polysaccharides such as glycogen and starch as stored fuel is that the oxidation of TGs yields twice as much energy as the oxidation of carbohydrates, since the carbon atoms of fatty acids are more reduced than those of sugars. Humans have adipose tissue under the skin, in the abdominal cavity and in the mammary glands. With 15 to 20 kg of TGs accumulated in their adipocytes, moderately obese people can meet their energy needs for months by using their fat stores. Most natural fats, such as vegetable oils, dairy products and animal fats, contain complex mixtures of simple and mixed TGs. These foods contain a variety of fatty acids that differ in chain length and degree of saturation. For example, vegetable oils, such as corn and olive oil, are composed of TGs that contain largely unsaturated fatty acids and are therefore liquid at room temperature.[1] Hypertriglyceridemia is a disorder of lipid metabolism resulting from excessive synthesis, defective processing and clearance, or both. The fact that hypertriglyceridemia has recently been identified as a risk factor for the development of cardiovascular disease (CVD) and has been known to play an important role in the etiopathogenesis of acute non-biliary pancreatitis and non-alcoholic steatohepatitis for years, reveals the necessity of its treatment. In recent prospective studies, the link between hypertriglyceridemia and CVD has been reported especially in Type 2 diabetics with low HDL-C and high LDL-C levels. In the presence of severe hypertriglyceridemia, eruptive xanthomas and lipemia retinalis may be seen in addition to acute pancreatitis.

In some cases, very high levels of CM can cause chylomicronemia syndrome, characterized by recurrent abdominal pain, nausea, vomiting and pancreatitis.

If a secondary cause cannot be identified, the disorder is classified as primary hypertriglyceridemia. Many genetic defects in TG metabolism may be responsible for primary hypertriglyceridemia. Ethnic factors may also play an important role in the development of dyslipidemia. Although primary causes account for a very small proportion of hypertriglyceridemia, they should be considered in cases of severe hypertriglyceridemia and family history should be questioned. Although molecular studies are required for definitive diagnosis, molecular studies can be performed in 5% of all cases. The main diagnosis is based on clinical and family history. The main primary causes are mixed hypertriglyceridemia (Type V hyperlipoproteinemia), familial hypertriglyceridemia (Type IV hyperlipoproteinemia), familial combined dyslipidemia and familial dysbetalipoproteinemia (Type III hyperlipoproteinemia). The main causes of secondary hypertriglyceridemia are obesity, diabetes mellitus, hypothyroidism, nephrotic syndrome, sedentary life, some drugs (beta-blockers, glucorticoids, estrogen, some immunosuppressive drugs, thiazide group diuretics, etc.) and some other diseases (Cushing's syndrome, rheumatoid arthritis, systemic lupus erythematosus, AIDS, Gaucher disease, Werner syndrome, sepsis, etc.).[3]

PRINCIPLES OF THE PROCEDURE

The test has colorimetric enzyme measurement method. TGs are enzymatically hydrolyzed to fatty acids and glycerol by lipoprotein lipase. Glycerol is phosphorylated by adenosine triphosphate (ATP) to form glycerol-3-phosphate (G-3-P) and adenosine diphosphate (ADP) under the catalysis of the enzyme glycerol kinase (GK). G-3-P is oxidized to dihydroxyacetone phosphate (DAP) by glycerol phosphate oxidase (GPO), producing hydrogen peroxide (H_2O_2). H_2O_2 reacts with 4-chlorophenol (4-CP) and 4-aminoantipyrine (4-AAP) in the presence of peroxidase to form a red dye called quinoneimine. The color intensity is proportional to the concentration of TG and is measured photometrically.

REAGENTS

Kit Contents

R1 4-chlorofenol (2,7 mM). 4-AAP (0,3 mM). ATP (2 mM). GK (> 1000 U/L). POD (> 1000 U/L). LPL (> 2000 U/L). GPO (> 5000 U/L). Good's buffer pH 7,20 (50 mM). Surfactants. Sodium azide (<0,1%).

Warnings and Precautions

IVD: For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.
Contains sodium azide.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA standards.

Danger

EUH032	: Releases a very toxic gas if contacts with acid.
H317	: May cause allergic skin reaction.

Precaution

P280	:Use protective gloves / clothes / glasses / mask.
P264	:Wash your hands properly after using.
P272	:Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention
P302+P352 :Wash with plenty of water and soap if it contacts with skin.
P333+P313 :Seek medical help if it irritates your skin or develops rash.
P362+P364 :Remove contaminated clothes and wash properly before using.
Disposal
P501 :Dispose the vials and contents according to the local regulations.

Reagent Handling

- Reagent cartridges should be kept in an upright position for at least 2 hours after receipt and before use to eliminate any air bubbles that may have formed inside.
- If the cartridge is dropped, it should be kept upright for 1 hours before use to allow the air bubbles to dissipate.
- Foam and air bubbles may form in the reagents; this may prevent accurate detection of the reagent level inside the cartridge, leading to insufficient reagent aspiration and potentially affecting the analysis results.

Reagent Storage [4]

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	+2/+8°C	Until expiration date	Store in upright position.
Onboard	System Temperature	60 days	

Indications of Reagent Deterioration

When a calibration error occurs or the control value falls outside the accepted range, the reagents may be considered to have deteriorated. In this case, the test results are not valid and the samples should be reanalyzed. Recalibration may be required.

Alternative Result Units

Use the unit conversion table provided below for alternative result units.

Default Unit	Conversion Factor	Result Unit
mg/dL	0.0113	mmol/L

SAMPLE COLLECTION AND PREPARATION FOR ANALYSIS

Sample Types

The recommended sample types for this test are listed below.

Sample Type	Collection Tube
Serum	Serum tubes
Plasma	Li-heparine sample collection tubes

Follow the instructions provided with your sample collection tubes for use and handling.

Sample Conditions

- To obtain reliable results, serum should be free of fibrin, blood cells, or foreign particles. In samples obtained from individuals receiving anticoagulant or thrombolytic therapy, fibrin residues may be observed due to incomplete clotting.
- To reduce the risk of contamination between samples, a new pipette or pipette tip should be used for each procedure.
- Non-hemolytic samples should be used.

Preparation for Analysis

- When preparing sample tubes, the manufacturer's instructions should be followed; separation by standing alone is not sufficient.
- Samples should be free of air bubbles. Before analysis, bubbles should be removed using a stick, and a separate stick should be used for each sample.
- If the samples contain fibrin, red blood cells, or other particulate matter, centrifuge them again before testing to ensure reliable results.
- If samples do not appear homogeneous, they should be mixed by low-speed vortexing or by inverting the tube 10 times.

Sample Storage [16]

Temperature	Maximum Storage Time
+2/+8°C	7 days [18]
+20/+25°C	2 days [18]
-20°C	3 months [19]

The information provided applies to serum and plasma samples. Repeated freeze–thaw cycles of samples should be avoided. [19]

PROCEDURE

Materials Provided
Triglycerides Reagent Kit
Materials Required but Not Provided

- Multiconstituent Calibrator (Lyophilized) or commercially available controls
- Saline solution (0.85%–0.90% NaCl) for sample dilution

Assay Procedure

- If primary or aliquot tubes are used, ensure that a sufficient amount of sample is available.
- To prevent evaporation of the specimen, make sure that the tube volume is adequate before each test.
- Minimum Sample Volume Requirements:
  - Sample volume for a single test: 1,5 µL
  - This volume does not include dead space or the additional aspiration volume.

Sample Dilution Procedures

This method demonstrates measurement linearity up to 1250 mg/dL. For samples with results above this value, the automatic dilution procedure of the autoanalyzer should be applied or manual dilution may be performed.

Automatic Dilution Protocol

The system dilutes the sample at a 1:10 ratio and automatically calculates the concentration by multiplying the result by the dilution factor.

Manual Dilution Procedures

Dilute the sample with a 0.85%–0.90% NaCl solution. Enter the dilution factor on the relevant screen of the device. If the user does not enter the dilution factor, the result must be manually multiplied by the correct factor before it is reported. If the result of the diluted sample is below 5 mg/dL, which is the lower limit of the measurement range, the result is not reported, and the procedure is repeated with an appropriate dilution.

Calibration

The use of a Lyophilized Multiconstituent Calibrator is required for this test.

Multiconstituent Calibrator- Lyophilized
Ref.No: 06R56-02

Calibration stability is 30 days. Changes in the reagent lot number require recalibration. Calibration is necessary if quality control results are not within acceptable limits.

Quality Control Procedures

Commercially available control materials with values assigned by this method may be used.

At least two levels of control should be run every 24 hours. Each laboratory should establish its own quality control procedures. If more stringent control monitoring is required, refer to the quality control procedures established for your laboratory.

EXPECTED VALUES

Each laboratory should investigate the transferability of expected values for its own patient population and, if necessary, establish its own reference range.

Reference Range [6.17]

	mg/dL	mmol/L
• Normal	: <150 mg/dL	<1,70 mmol/L
• Borderline High	: 150-199 mg/dL	1,70-2,25 mmol/L
• High	: 200-499 mg/dL	2,26-5,64 mmol/L
• Very High	: ≥ 500 mg/dL	≥ 5,65 mmol/L

Triglyceride measurement should be performed after 12–16 hours of fasting. Currently, the NCEP Adult Treatment Panel (NCEP-ATP)

recommendations for lipid-lowering therapy are accepted worldwide. The NCEP recommends that a fasting lipid panel (including LDL-C, HDL-C, and TG) be performed beginning at 20 years of age and repeated every 5 years. If a significant risk factor is present, the test should be repeated annually. If the TG concentration exceeds 150 mg/dL, the measurement may be repeated after a fasting period. If available, Apolipoprotein B (ApoB) and Lipoprotein(a) [Lp(a)] measurements may be performed to assess the risk of atherosclerosis in patients with hypertriglyceridemia.[3]

Precision

Sample	n	Mean (mg/dL)	Within-Run (Repeatability)[10,11] SD	Within-Run (Repeatability)[10,11] %CV	Within-Laboratory (Total)*[10,11] SD	Within-Laboratory (Total)*[10,11] %CV
Level 1	80	100.4	1.28	1.27	3.93	3.91
Level 2	80	239.4	2.34	0.98	7.06	2.95

*: Includes within-run, between-run, and between-day variability.

Lower Measurement Limits

The lower measurement limits refer to the range between the lower limit of quantitation (LLoQ) and the upper limit of quantitation (ULoQ), within which the analyte concentration can be measured with the required medical and laboratory accuracy without dilution, concentration, or any pre-treatment.[7] The LoD and LoQ values have been verified using the CLSI EP17-A2:2012 protocol.[8]

	mg/dL
LoD**	3.2
LoQ***	5

**** :** The LoD represents the lowest concentration at which the analyte can be detected with 95% probability, based on ≥ 60 replicates of samples with low analyte levels.
*****:** When determining the LoQ value, a coefficient of variation (%CV) of ≤20% is used as the basis.

Measuring Interval

This method shows measurement linearity in the activities between 5-1250 mg/dL. For samples with results above this value, the automatic dilution procedure should be applied. For additional information, please refer to the instrument's user manual. Linearity studies data have been verified by using CLSI EP06-A:2003 protocol.[9]

Interference

Endogenous interferant and analyte concentrations that have been used in the Triglyceride scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[13,14]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from Triglyceride interference scanning test is appropriate, is determined as ±10%.[15]

In Triglyceride test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Interferant-Concentration	Triglyceride Target (mg/dL)	n	Observed Recovery %
Hemoglobin 315 mg/dL	162.0	3	104
Bilirubin 4.97 mg/dL	171.9	3	103
Ascorbic acid 9.9 mg/dL	158.5	3	100
Total protein 15 g/dL	165.8	3	108

*In calculating how many times the control and test samples (containing interferent), prepared as a serum pool, would be run repetitively, the within-run SD values previously determined for the relevant method and the total allowable error rate defined as the interference limit were used. In the calculations, a type 1 error (α error) rate of 5% and a type 2 error (β error) rate of 10% (90% power) were accepted.[14]

It should be noted that, in addition to endogenous interferents, various drugs and metabolites; anticoagulants (e.g., heparin, EDTA, citrate, oxalate) and preservatives (e.g., sodium fluoride, iodoacetate, hydrochloric acid); substances that may come into contact with samples during collection and handling (such as serum separation devices, sample collection containers and their contents, catheters, catheter flushing solutions, skin disinfectants, hand sanitizers and lotions, glassware detergents, powdered gloves, etc.); dietary substances known to affect certain tests (e.g., caffeine, beta-carotene, poppy seeds); or certain substances present in a sample that may cause interference due to foreign proteins (e.g., heterophilic antibodies), autoimmune responses (e.g., autoantibodies), or malignancy (e.g., paraproteins causing interference in phosphate tests and indirect ion-selective electrode methods) may lead to various interferences and erroneous evaluations for many different reasons.[14]

These performance characteristics were obtained using an autoanalyzer. Results may vary when different equipment or manual procedures are used.

Method Comparison

As a result of the statistical evaluation of method comparison data, the table was constructed according to the Passing–Bablok regression method as follows:

Archem Triglyceride – Roche Cobas Integra 400 Plus

	Units	n	Correlation Coefficient	Intercept	Slpoe	Concentration Range
Serum	mg/dL	100	0.9990	0.69	0.98	8-1198

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests